EUSTAR biobanking: recommendations for the collection, storage and distribution of biospecimens in scleroderma research

Beyer, C ; Distler, J H W ; Allanore, Y ; Aringer, M ; Avouac, J ; Czirják, L ; Cutolo, M ; Damjanov, N ; Del Galdo, F ; Fligelstone, K ; Guiducci, S ; Kowal-Bielecka, O ; van Laar, J M ; Martucci-Cerinic, M ; Müller-Ladner, U ; Riemekasten, G ; Tarner, I H ; Tyndall, A ; Kennedy, A T ; Valentini, G ; Vettori, S ; Walker, U A ; Denton, C ; Distler, O

Abstract: The European League Against Rheumatism Scleroderma Trials and Research Group (EUSTAR) has established an online database with clinical data of currently more than 8200 patients with systemic sclerosis (SSc). In addition to clinical research, EUSTAR fosters biomolecular studies to develop novel biomarkers and therapies for SSc. High-quality biospecimens are the basis for successful biomolecular studies. The EUSTAR biobanking group has therefore developed recommendations to standardise the collection, storage and distribution of SSc biospecimens at EUSTAR centres. These recommendations consider the scientific challenges associated with biomolecular research in SSc and the organisational requirements of EUSTAR. They were approved by the EUSTAR executive committee as well as the EUSTAR board. Once they become effective, these recommendations will be the basis for international EUSTAR studies with large numbers of SSc biospecimens. These recommendations might also be followed by other SSc consortia to enable exchange of biosamples between different SSc initiatives and might serve as a template for biobanking initiatives in other rheumatic diseases.

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EUSTAR biobanking: ***Recommendations for the Collection, Storage, and Distribution of Biospecimens in Scleroderma Research***

Christian Beyer[1], Jörg H.W. Distler[1], Yannick Allanore[2], Martin Aringer[3], Jérôme Avouac[1,2], László Czirják [4], Maurizio Cutolo[5], Nemanja Damjanov[6], Francesco Del Galdo[7], Kim Fligelstone[8], Serena Guiducci[9], Otylia Kowal-Bielecka[10], Jacob M. van Laar[11], Marco Martucci-Cerinic[9], Ulf Müller-Ladner[12], Gabriela Riemekasten[13], Ingo H. Tarner[11], Alan Tyndall[14], Ann Tyrrell Kennedy[8], Gabriele Valentini[15], Serena Vettori[15], Ulrich A. Walker[14], Christopher Denton[16] and Oliver Distler[17]

From the EUSTAR biobanking group

[1]Department of Internal Medicine 3 and Institute for Clinical Immunology, University of Erlangen-Nuremberg, Erlangen, Germany

[2]Rheumatology A Department, René Descartes University, Paris, France

[3]Department of Rheumatology, Carl-Gustav-Carus University Dresden, Dresden, Germany

[4]Department of Immunology and Rheumatology, University of Pécs, Pécs, Hungary

[5]Research Laboratory and Academic Unit of Clinical Rheumatology, Department of Internal Medicine, University of Genova, Italy

[6]Institute of Rheumatology, University of Belgrade, Belgrade, Serbia

[7]Section of Musculoskeletal Disease, Institute of Molecular Medicine, St James's University Hospital Leeds, Leeds, UK

[8]Federation of the European Scleroderma Associations (FESCA)

[9]Department BioMedicine, Division of Rheumatology, University of Florence, Florence, Italy

[10]Department of Rheumatology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland

[11]Musculoskeletal Research Group, Institute of Cellular Medicine, Newcastle University, Newcastle, UK

[12]Department of Internal Medicine and Rheumatology, Justus-Liebig-University Giessen, Giessen, Germany, and Department of Rheumatology, Clinical Immunology, Osteology and Physical Medicine, Kerckhoff-Klinik, Bad Nauheim, Germany

[13]Department of Rheumatology and Clinical Immunology, Charité Universitätsmedizin Berlin, Berlin, Germany

[14]Department of Rheumatology, Basel University, Basel, Switzerland

[15]Rheumatology Department, Second University of Naples, Naples, Italy

[16]Rheumatology Department, Royal Free Hospital London, London, UK

[17]Center of Experimental Rheumatology and Center of Integrative Human Physiology, Department of Rheumatology, University Hospital Zurich, Zurich, Switzerland

Correspondence and reprints request to:

Oliver Distler, MD, Department of Rheumatology, University Hospital Zurich, Gloriastr. 25, 8091 Zürich, Switzerland, Email: Oliver.Distler@usz.ch, Tel: ++41-44-255-2932

Abstract

The EULAR scleroderma trials and research group (EUSTAR) has established an online database with clinical data of currently more than 8,200 patients with systemic sclerosis (SSc). In addition to clinical research, EUSTAR fosters biomolecular studies to develop novel biomarkers and therapies for SSc. High-quality biospecimens are the basis for successful biomolecular studies. Therefore, the EUSTAR biobanking group has developed recommendations to standardize the collection, storage, and distribution of SSc biospecimens among EUSTAR centres. These recommendations consider the scientific challenges associated with biomolecular research in SSc as well as the organizational requirements of EUSTAR. They were approved by the EUSTAR executive committee as well as the EUSTAR board. Once they become effective, these recommendations will be the basis for international EUSTAR studies with large numbers of SSc biospecimens. These recommendations might also be followed by other SSc consortia to enable exchange of biosamples between different SSc initiatives and might serve as a template for biobanking initiatives in other rheumatic diseases.

Introduction

Systemic Sclerosis (SSc) is an orphan disease with a prevalence of approximately 1 in 12,000 [1]. The course of disease is highly variable and patients with SSc may experience spontaneous regression of fibrosis. This places biomolecular research in a dilemma: because of the variable course of the disease, researchers need high numbers of SSc biospecimens to identify predisposing genetic factors, establish biomarkers, or develop novel treatment strategies. The low prevalence of SSc, however, limits the size of study cohorts at local and regional medical centres. Thus, researchers can only establish large study cohorts with high numbers of SSc biospecimens by nationwide and international cooperation. In this context, the EULAR Scleroderma Trials and Research group (EUSTAR) has initiated a unique biobanking platform for the collection, storage, and distribution of biospecimens in an international network of SSc research centres.

The Organisation for Economic Co-operation and Development (OECD) first defined the term 'biobank' as 'a collection of biological material and the associated data and information stored in an organized system for a population or a large subset of a population' [2, 3]. Biological material from patients with SSc is the direct source of biomolecular research. Regarding the high sensitivity of new biomolecular technologies, the reliability of SSc research depends on the quality and consistency of the specimens. The lack of standardized, high-quality biospecimens is a major roadblock in biomolecular research [4]. Therefore, the EUSTAR biobanking group has developed recommendations and quality standards for collection, storage, and distribution of SSc biospecimens (Online supplement 1). These recommendations consider scientific challenges associated with biomolecular research in SSc as well as organizational requirements of EUSTAR. They rely on state-of-the-science approaches and concepts of good laboratory practice and are reviewed on a regular basis. If

all participating SSc research centres achieve these quality standards, EUSTAR biobanking will provide a framework for pivotal studies.

1. Collection and Processing

In addition to documenting a minimal essential data set (MEDS), acquisition and storage of serum samples, whole blood samples, and skin biopsies from patients with SSc are strongly encouraged in the EUSTAR cohort. Prior to data assessment and biospecimen collection, each patient must give informed consent. Local or national ethics committees approve informed consent forms for each EUSTAR centre. These forms independently address data assessment and biospecimen collection according to national laws. Apart from other types of biospecimens, the collection of DNA samples requires separate informed consent.

For all kinds of biospecimens, it is important to maintain a cold chain from the point of collection through processing and storage. Biosamples should be kept on ice and the centrifuge should be cooled. In addition, collection and processing should be performed under sterile conditions using sterile needles, pipets and other instruments as well as a sterile hood to avoid contamination. Appropriate aliquoting of biospecimens helps to avoid repetitive freeze and thaw cycles, which can damage the integrity of samples [5, 6]. Online supplement 2 provides a detailed guide on processing of SSc blood, serum, and skin samples.

2. Storage

In EUSTAR biobanking, specimens are stored in the repositories of the affiliated research centres. Therefore, standardization of storing procedures and equipment among EUSTAR centres is crucial to ensure high quality and consistency of SSc biospecimens. In general,

selection of storage equipment depends on the type of specimens, the anticipated storage time, and the intended use of the samples [6, 7].

Serum and (whole) blood samples as well as tissue samples for protein and RNA analysis should be stored at -80°C as provided by special mechanical freezers. Temperatures of -80°C or lower may be necessary for the stability of certain cytokines, such as TNF-α [5, 6, 8]. Furthermore, freezing temperatures of -80°C are adequate for successful preservation of tissues for extended periods of time [9]. If a -80°C freezer is not available, EUSTAR serum and blood samples can be stored at -20°C but need to be labeled appropriately. Independent from the type of specimen, proteomic techniques require very-low temperature storage conditions as provided by liquid nitrogen freezers. In general, screw-cap cryovials should be used for long-term, low-temperature storage; glass vials or vials with popup tops are not suitable. Finally, paraffin blocks should be stored at room temperature (not exceeding 27°C) in an area with pest and humidity control [5, 6, 8, 10].

For any storage equipment, acceptable temperature ranges should be determined before the equipment is put into service. Temperature ranges allow for operating variations and provide some variation for warming when the biospecimens are accessed. Of note, temperature probes measure the temperature where the probes are located; therefore, different locations in the equipment might exhibit different temperatures. Freezers that are full will likely display temperature readings that are different from readings of empty equipment [5, 6, 10-12].

Loss of electrical power or failure of freezers can lead to warming and destruction of the biospecimens. The length of time that results in the significant warming of the stored material will vary by the properties and temperature of the material (thermal loading), the ambient conditions, and the design of the unit [5, 13]. To bridge transient loss of commercial utility

power, EUSTAR repositories should be connected to an uninterruptible power supply (UPS), such as a “battery backup”. Apart from storage equipment, computers and electronic systems, such as environmental monitoring systems, safety systems (e.g., oxygen sensors, ventilations systems, etc.), or controllers for freezers, should also be protected by an UPS. In addition, adequate backup capacity for low temperature units is necessary for possible failure of storage equipment. The total amount of backup storage required for EUSTAR repositories is determined empirically, but will typically be 10 % for mechanical freezer storage [5, 6]. Distributing aliquots to different low-temperature units can also prevent the total loss of single batches upon equipment failure. Special issues must be addressed when using liquid nitrogen freezers (online supplement 3).

In EUSTAR repositories it is critical to maintain ambient temperature. For optimal life of mechanical freezers, repository ambient temperatures should be maintained between 15°C and 22°C. In addition, sufficient air circulation prevents excess moisture and condensation. Left unchecked, excess humidity can lead to fungal growth, which may affect specimen integrity and cause health problems for staff. Sufficient space for air circulation prevents excess heat accumulation in areas where freezers and refrigerators are employed. Lighting in EUSTAR repositories should provide a safe working environment and allow materials to be accurately put away and retrieved [5, 6].

Repositories should employ basic security systems to ensure protection of the specimens. The systems should be monitored and alarms responded to 24 hours per day. An individual should be available at all times to respond to an alarm in a time frame that minimizes loss or damage to the stored materials [5, 6, 8]. Storage units with defined environmental conditions have temperature-monitoring devices that can be visually inspected on a regular basis. In addition, automatic temperature monitoring systems continually monitor temperatures of all critical

equipment and generate alarms. The alarm notification system should call or page the individual “on call” (or should activate the “on call” list) rather than simply providing passive notification [5, 6, 8].

A system for preventative maintenance and repair of storage equipment and facilities should be in place. EUSTAR repositories should perform system maintenance at regular intervals per manufacturer’s recommendation. Essentially all equipment comprised of multiple components and exposed to various environmental conditions wears out with time. Routine assessments and maintenance may significantly extend the lifetime of equipment. Well-qualified personnel with expertise in monitoring and repairing repository equipment (especially freezers and refrigerators) should perform regular and emergency repairs. Finally, EUSTAR repositories should establish security systems that limit access to appropriate staff and protects against physical intrusion from unauthorized individuals [5].

3. Biological material tracking

EUSTAR centres should install effective tracking systems to track specimens from the site of collection to their arrival in the repository as well as subsequent shipment to other centres. Critical components of these systems include unique specimen identifiers (ID), appropriate specimen labels, and inventory systems for specimen tracking. Each specimen container should receive a label that tightly adheres under all projected storage conditions. Information encoded on labels should be resistant to all common laboratory solvents. If possible, labels should be computer-printed. Human specimens should be labeled in a way that protects privacy as well as confidentiality and is in compliance with applicable laws and institutional policies [5, 6]. To link SSc biospecimens with the MEDS online database, EUSTAR recommends to generate the specimen ID as follows: EUSTAR center number - EUSTAR

patient number – date of collection - aliquot number (e.g., EUSTAR center 001, patient number 075, collected on May 10th, 2009, aliquot number 002: 001-075-100509-002).

In addition to sample tracking, EUSTAR has developed an online records management system that permits detailed records of the collection, processing, and distribution of specimens. The system should also track significant events such as thaws, loss, and destruction of serum samples. Furthermore, records should include training documents, protocols, informed consent documentation, procurement documentation, processing records, equipment maintenance, specimen storage location information, sample distribution, and quality control activities. Security systems should ensure confidentiality and security of all stored records. Access to records should also be restricted on a "need to know" basis. A policy should be in place for the destruction or return of records that no longer need to be retained [4-6].

4. Retrieval

Retrieval of specimens for analysis or shipment requires strict adherence to protocols for proper specimen inventory and tracking. First, the location of specimens should be verified. A specimen requisition is generated and checked for accuracy before transmission to the repository. Specimens should be located and pulled from storage as documented on specimen requisition forms. When frozen biospecimens are shipped to other EUSTAR centres, a cold chain should be maintained to avoid warming and thawing of biospecimens [5, 6].

5. Packaging and shipping

Prior to shipment, the specifications for the biological material should be determined; this includes regulatory and physical requirements necessary to ensure proper shipping conditions. Packaging and shipping should conform to all governing regulations. Air shipments should conform to the International Air Transport Association (IATA) standards, and ground shipments to applicable national standards. International Society for Biological and Environmental Repositories (ISBER) best practices and IATA regulations provide information on international transport regulations and sample classification for shipment [5, 6, 12, 14]. Online supplement 4 provides detailed information on technical and regulatory issues of biosample packaging and shipping.

6. Legal and ethical issues

Appropriate annotation of biospecimens is crucial to the overall usefulness of EUSTAR biobanking as a tool for scientific research. In addition to technical considerations relating to the physical quality of a biospecimen, multiple ethical and legal issues relate to biospecimen collection. In this context, EUSTAR biobanking follows highest ethical standards and addresses concepts of good clinical and laboratory practice; this includes respecting the autonomy of research participants, protecting research participants from breaches of privacy and confidentiality, developing appropriate policies for biospecimen use, and ensuring scientifically sound research [5, 6].

On an international level, the collection and use of biospecimens is regulated by an amalgam of differing and occasionally conflicting local and international laws and policies. Thus, EUSTAR repositories proceed carefully, not only in their daily work, but also with respect to international exchange of samples and associated data. Each EUSTAR center needs to operate with approval of the relevant ethics committee. Prior to approval, ethics committees evaluate

the processes for collection, storage, distribution, and use of SSc biospecimens in the repository [5, 6].

Informed consent is mandatory before patients can be included in the EUSTAR cohort and before biosamples can be collected. Informed consent is a process that offers subjects sufficient information to make an informed choice about whether to provide specimens and data to the EUSTAR centre and agree to future research use. Informed consent information should be as specific as possible and provide information about the scientific goals of EUSTAR. This should include future analysis of molecules and biomarkers that are still unknown at the time of collection, if required by the ethical committee [5, 6, 15, 16]. EUSTAR believes it was unethical to not use existing biomaterials for this purpose, as long as patients' will or well-being would not be jeopardized. Finally, online supplement 5 addresses in detail the content of EUSTAR informed consent forms.

Internal (i.e., by EUSTAR centres) as well as external (e.g., by other research centres or pharmaceutical companies) requests for EUSTAR SSc biospecimens and MEDS data will undergo scientific review by the according EUSTAR committee and the EUSTAR board. EUSTAR has published strict rules to apply for, review and decide on scientific projects (online supplement 6). Once biospecimens and clinical data are distributed to the investigators, their use restricted to the approved study. Further analysis requires new EUSTAR approval.

Finally, SSc biospecimens (including DNA) and clinical data are the property of the patient and the EUSTAR centre that has delivered it. In this context, EUSTAR repositories address formal and continuing responsibility for custodianship of collected biospecimens and associated data. The repositories have to ensure the physical integrity of biospecimens and

clinical data. Thus, EUSTAR centres - as well as individual patients - may withdraw their biosamples and clinical data from a specific project at any time [6].

7. Standardized protocols, quality control and quality assurance

To achieve high levels of intrinsic quality and comparability in EUSTAR research, the definition and publication of biobanking recommendations are an essential starting point. Based on these recommendations, EUSTAR centres develop standardized protocols for collecting, storing, and distributing SSc biospecimens adapted to local requirements. These protocols should be written by an individual or group of individuals with experience in successfully performing the processes described. Effective protocols are reviewed on a regular basis [4-6].

Quality assurance (QA) and quality control (QC) policies are developed by repositories to minimize errors that could adversely affect scientific results. QA and QC policies encompass equipment maintenance and repair, training records, data management, record keeping, and adherence to protocols [4-6]. In addition, QC examination of biospecimens designated for research should be performed according to research protocols; this includes molecular quality control which characterizes nucleic acids and proteins. Cost effective approaches for tissue resources require simple methods of quality control, which can be expanded per investigator request [5, 17].

EUSTAR repositories should be subjected to regular audits [5, 6]. EUSTAR prefers independent auditors from external institutions. Audits focus on adherence to the recommendations presented herein. At the end of each audit, centres receive a list of deficiencies which they have to remedy within 3 months. If not accomplished, these centres

will be –at least temporarily- excluded from the EUSTAR cohort. Paying tribute to high costs of audits, approximately 10 % of centers actively contributing to biobanking will be randomly chosen to receive audits each year. This does not apply to EUSTAR centres that are already subject to regular external audits covering all necessary procedures for EUSTAR biobanking.

8. Training

To ensure the collection of high-quality SSc biospecimens, personnel are well qualified and trained to adhere to applicable protocols. Proper training promotes quality in specimen handling, good ethical practices, and compliance with appropriate policies and regulations of the repository. All repository staff should be adequately trained to perform the tasks required by their particular position. Support for training is essential for implementation of certain tasks and in some cases might require additional resources or time off from regular responsibilities. To ensure quality in repository activities employee performance should be routinely monitored to identify needs for additional training [5, 6].

Federal law mandates the training of medical personnel who obtain informed consent and collect SSc biospecimens as well as clinical data. Without interfering with federal regulations, EUSTAR recommends additional yearly instructions for medical personnel to emphasize the needs for sound and successful SSc research. Except some areas of safety, training of personnel involved in the processing, storage, and retrieval of SSc specimens and data is less tightly regulated by federal law. These personnel should receive theoretical and hands-on training at least once a year. Each EUSTAR centre develops written training plans that cover all important biobanking procedures and consider local circumstances. EUSTAR will provide templates for these training plans. At the end of each training, participants should pass tests

with the results being recorded. Independent from position and tasks, all new employees should receive training before they start working.

Each EUSTAR repository should have an Individual Responsible for Training (IRT) who monitors and documents training, and maintains records of employees. The IRT will coordinate safety training with the repository's safety officer as well as with other individuals responsible for specific areas of repository procedures (e.g., shipping and handling). Finally, the IRT determines trainers for selected working areas. Trainers regularly perform the procedures in question, have completed the training program previously, and are skilled in explaining the elements of the task. If necessary, the IRT may hire external trainers for special training programs or send staff to external training courses [5, 6]. In this context, EUSTAR organizes a biobanking workshop at the educational EUSTAR course for young researchers and physicians every other year.

9. Safety

Issues related to safe operation of repositories are complex and depend on the particular activities. National, regional or local statutes typically cover regulations governing safety. Each EUSTAR repository should determine affected areas of safety and develop a safety program to protect its employees. EUSTAR repositories should address biological, chemical, electrical, and fire safety issues. All human specimens, are potential biohazards and should be treated with precautions. Individuals should be trained in the possible hazards and should take precautionary measures. For example, staff members working with human patients are encouraged to be vaccinated against hepatitis. In addition, personal protective wear, e.g. eye-shades or gloves, can lower the risk for contamination with hazardous material [5, 6, 8].

Concluding remarks

The EUSTAR cohort already harbors clinical data on more than 8,200 patients with SSc. This offers a unique chance to study the course of SSc and the various disease manifestations. With the implementation of the EUSTAR biobanking project, EUSTAR enters a new era of biomolecular and translational research in the field of SSc. The collection of biospecimens from a large number of SSc patients will help to establish novel biomarkers and develop effective therapies. The EUSTAR recommendations for biobanking are the basis for successful biomolecular studies applying high-sensitivity techniques. Obviously, these recommendations should not be a barrier to the participation in EUSTAR if not all points are totally satisfied. Nevertheless, only if all participating EUSTAR centres adapt these recommendations in daily laboratory routine, EUSTAR biobanking will pave the way for biomolecular landmark studies, which can change our understanding of SSc. For this reason, EUSTAR defined mandatory requirements to participate in EUSTAR biobanking, which are summarized together with other important recommendations in Table 1. Other SSc consortia might also follow these recommendations to enable exchange of biosamples between different SSc initiatives. Notably, there are no detailed recommendations published for establishing biorepositories in rheumatic diseases. Thus, the EUSTAR biobanking recommendations might also serve as a template for biobanking initiatives in other rheumatic diseases.

Glossary:

BIOBANKING – The process of storing biomaterial or specimens for future use.

BIOBANK / BIOREPOSITORY –An entity that receives, stores, processes, and disseminates biological material.

BIOHAZARD – An organism or substance derived from an organism that poses a threat to human health. This includes medical waste, samples of a microorganism, virus or toxin from a biological source.

CUSTODIAN – The individual responsible for the management of a biospecimen resource. The custodian works with other key stakeholders in the management of the resource including the tracking of all relevant documentation for the resource and for ensuring that policies regarding access to the resource are in place and implemented according to appropriate recommendations.

DRY ICE – Solid phase carbon dioxide (CO_2). CO_2 solidifies at -78.5 °C.

LIQUID NITROGEN – Coolant used to cool and store samples. Nitrogen becomes liquid at -196°C. Samples stored in the vapor phase of liquid nitrogen are -190 °C and warmer, depending on the distance from the liquid phase.

QUALITY – Conformance of a specimen or process with pre-established specifications or standards.

QUALITY ASSURANCE (QA) – An integrated system of management activities involving planning, implementation, documentation, assessment, and improvement to ensure that a process or item is of the type and quality needed for the project. Same as Quality Management System (QMS).

QUALITY CONTROL (QC) – Specific tests defined by the QA Program to be performed to monitor procurement, processing, preservation and storage; specimen quality; and test accuracy.

Suppl. 1. Development of recommendations for EUSTAR biobanking

Since it is a central objective of EUSTAR to stimulate international studies with large numbers of SSc patients and biospecimens, the EUSTAR board initiated a biobanking project for collection, storage, and distribution of biospecimens among participating centres. For economic reasons, the EUSTAR board decided on a decentralized structure: EUSTAR centres collect and store biospecimens of their patients; central recording and tracking of SSc biospecimens via the EUSTAR online database facilitates the distribution of SSc specimens and data for research. To coordinate and standardize this biobanking project, the EUSTAR board nominated EUSTAR members to form a biobanking group. The first task of this group was to develop recommendations for biosample collection, storage, and distribution. When developing these recommendations, the biobanking group had to consider both high quality of future biosamples and feasibility of recommended procedures: as an international organization EUSTAR faces wide ranges of technical and economic resources among its participating centres. So far, the EUSTAR biobanking group focused on the collection, processing, and storage of serum samples, blood samples and skin biopsies, since these types of biospecimens are most important in SSc research. In the future, however, EUSTAR may extend the recommendations to other SSc biospecimens. Once published, the recommendations will become officially binding for centres participating in EUSTAR biobanking.

Suppl. 2. Collection of SSc biospecimens

a. blood and serum samples

For collection of serum and whole blood samples, a trained phlebotomist takes blood from a peripheral vein of the patient together with routine blood work to avoid discomfort to the patient. For scleroderma patients in particular, blood samples should be taken at comfortable warm room temperatures, and a small needle size should be used. Since the time between phlebotomy and processing is crucial for stability of many analytics, blood samples should be kept on ice and processed within 2 hours after phlebotomy [8].

i. For serum samples, about 10 ml of blood, which is equivalent to 3-4 ml serum, should be obtained from each patient. After blood coagulation and centrifugation for 10 min at 400 *g*, serum can be obtained as cell free supernatant in the upper phase; contamination with the lower cellular phase must be avoided. Serum should be aliquoted into sterile cryovials, labeled, snap-frozen, and stored at -80°C [5, 6, 8]. EUSTAR recommends an aliquot size of 500 µl.
ii. Whole blood samples are a source for genetic studies. About 5 ml of blood are collected in an EDTA tube, which inhibits blood coagulation. After snap freezing, whole blood samples should be stored at -80°C [5, 6, 8]. Special collections kits that stabilize nucleic acids can improve the outcome of DNA purification (e.g., PaxGene® tubes).
iii. Serum and blood samples for proteomics research require special handling since these techniques are more susceptible to external variations. After collection, samples are placed on ice throughout the whole processing [18]. Biospecimens should be processed with 1 hour after venipuncture. Snap-freezing and storage in liquid nitrogen at temperatures of -140°C are strongly recommended for proteomic techniques [18]. Samples that fulfill requirements for proteomics should be labeled adequately.

b. skin biopsies

When collecting skin biopsies for SSc research, a lesional (clinically involved) and a non-lesional (clinically uninvolved) area of skin are generally sampled. The anatomical site of the

biopsy should be recorded, and biopsies distal to the wrist should be avoided as healing may be very slow. Dermal thickness at the site of biopsy should be recorded according to the modified Rodnan Skin Score. All biopsies are performed with aseptic techniques: This includes the use of sterile punch biopsies and other instruments as well as thorough cleaning of the skin with antiseptic agents. After collection, skin biopsy samples should be processed within 30 min to minimize the effect of hypoxia upon genetic expression and the degradation of RNA and other tissue constituents [18-22]. If this is not possible, the specimens should be put on ice until dissection, and processing time should be recorded. Depending on the research purposes, biopsies are transferred to (i) tubes with formaldehyde for histology, (ii) cryovials with RNA stabilizing solutions such as RNAlater, or (iii) empty cryovials for protein analysis. Larger biopsies can be cut into two to three pieces for further analysis (e.g., one third for each histology, RNA and protein analysis).

i. For histology, skin biopsies are fixed in 10 % neutral buffered formalin for 6 to 24 hours depending on the size of the biopsy. Thereafter, samples can be embedded in paraffin following standard protocols. Finally, paraffin blocks are stored at room temperature not exceeding 27°C [5, 6, 8, 11].

i. Protein samples in screw-cap cryovials are snap-frozen in liquid nitrogen and then stored at -80°C. In this context, skin specimens should not come in direct contact with liquid nitrogen. Slow freezing must be completely avoided, as it results in the formation of ice crystals [5, 6, 8].

ii. After transferring skin samples in cryovials with RNAlater, samples for RNA analysis are stored at -80°C [5, 6, 8].

Suppl. 3. Special considerations for liquid nitrogen freezers

In general, vapour phase storage is preferred over storage in the liquid phase. Storage in the vapour phase avoids safety hazards inherent in liquid phase storage. Liquid nitrogen expands to 700 to 800 times of its original volume when brought to a gaseous phase at room temperature. This situation may produce explosion hazards. Glass, metal, and some plastic containers can explode if liquid nitrogen is trapped inside the container during removal from the freezer. Any container used or stored within these ultra low temperatures should be rated for these conditions. Where liquid nitrogen refrigeration is employed, an adequate supply of refrigerant should be maintained [5, 13]. Because nitrogen displaces oxygen, liquid nitrogen freezers require careful handling. Oxygen level sensors should be employed, and self contained breathing apparatus should be available for use in a "whiteout" condition [5]. Because of the low temperature, eye protection to protect against splashes is mandatory. Face and ear protection is recommended and heavy gloves should be worn over protective laboratory gloves. Backup capacity for liquid nitrogen freezers should amount to 3% of the total freezer capacity [5, 13].

Suppl. 4. Packaging and shipping of SSc biospecimens

Specimens may be exposed to temperature fluctuations during transit. Shipments of material that are subject to cold chain management are shipped with sufficient refrigerant to maintain temperature throughout the shipping cycle, with allowance for at least a 24-hour delay in arrival time. Serum, blood, and skin samples should be shipped at -70°C on dry ice. Dry ice employed for frozen shipments is a hazardous material requiring appropriate labeling. For proteomics research, dry nitrogen shippers should be used for the shipment of biospecimens. The IATA considers these shippers as non-dangerous products, since liquid nitrogen is fully absorbed in a porous material. Finally, paraffin blocks and slices can be shipped at ambient

conditions of 20 to 30°C; insulated packaging minimizes the effect of temperature fluctuations and protects the blocks from temperatures higher than 27°C [5, 6].

Packaging may be tested prior to use with specimens, and tests should include measuring all parameters that could influence specimen integrity, including temperature, light sensitivity, and structural quality. Shipments of specimens with critical temperature requirements should include a temperature-recording device that can verify the temperature of the material throughout the transport cycle. In some situations, repositories may first send a test shipment, which informs the shipper as to the adequacy of packing coolants [5, 6].

Shipments should be initiated when there are at least two working days left in the week, in case it does not arrive on the day it is scheduled for delivery. Both shipper and recipient should track all packages while in transit. The recipient should confirm that he will be able to receive the package before the shipper releases the shipment. The shipper should provide a 24-hour emergency contact for all packages transporting dangerous goods. The shipper should send a shipping manifest (preferably electronic) to the recipient prior to the release of the shipment. Confirmation of receipt and the condition upon arrival should be obtained for every shipment coming to or leaving a repository. A form that collects this information is sent with the shipment [5, 6]. Of note, many professional couriers experienced in the shipping of biomaterials fulfill these requirements.

EUSTAR repositories should maintain a shipment log to record the receipt and dissemination of shipments. Each shipment entry is given a unique number. The log should track the following elements: shipment/invoice number, recipient/source, date received or shipped, courier name and ID for tracking package, sample description, number of samples, study name and number if available, shipping conditions (e.g., dry ice, temperature, etc.), key

investigator name, signature of individual receiving the specimen, any discrepancies between the shipping manifest and the actual shipment [5, 6].

Suppl. 5: Informed consent forms

Each EUSTAR centre creates informed consent forms that need to be approved by local ethic committees prior to the collection of biospecimens and clinical data. Informed consent forms are written in the national language and adapted to national and local laws and regulations. Informed consent addresses collection, storage and scientific use of i) clinical data (MEDS), ii) biospecimens other than DNA, and iii) DNA for genetic studies. In this context, consent/patient signatures should be obtained separately for each item. This allows patients, who - for example - feel confident about providing clinical data, but not DNA, to contribute to the EUSTAR cohort. This also allows centres to participate in EUSTAR, even if they are not allowed to collect and send DNA according to national laws.

Adapted from Hansson [16], informed consent forms include the following points:

a. Goals of EUSTAR and purpose of the research. It needs to be specifically mentioned that biospecimens can be analyzed for molecules and biomarkers in the future that are still unknown at the time of collection.

b. Storage procedures and duration, which vary for both samples and data among EUSTAR centres because of different national laws. Safety measures to protect the integrity of biospecimens and clinical data should be described briefly.

c. The person who is responsible for the biobank and the identity of the data controller.

d. Distribution and sharing of clinical data and biospecimens only with authorized persons (from both academic institutions and industry). Of note, this may vary among EUSTAR centres because of different national laws. In this context, there are striking differences in how the distribution of DNA samples is regulated.

e. Anonymisation of biospecimens and clinical data.

f. Withdrawal of informed consent at any time without specifying any reason.

Suppl. 6: Requests for EUSTAR biospecimens and MEDS data (also see www.eustar.org)

a. General Rules

- EULAR Scleroderma Trials and Research group (EUSTAR) must be acknowledged on every presentation/publication that results from accepted proposals.
- EUSTAR centres providing samples and clinical data should be co-authors on manuscripts and presentations derived from the study (at least one per center) whenever possible. In case of a too high number of authors for the target journal, an adequate acknowledgement must be provided in the paper. Conflicts of interests in this matter will be decided by the Executive Officers. If submitting centres do not agree with this procedure, they must clearly state this in the proposal. There are no exceptions from this rule. The sequence of authors is decided by the submitting center, but should in general be based on the numbers of samples contributed to the study.
- The intellectual content of the proposal belongs to the submitting center. Misuse of the proposal for own studies is strictly forbidden.

b. Rules for submission of projects to Committees

- EUSTAR will allow also non members to apply. The work must be then published under EUSTAR committee overview and approval.
- EUSTAR submission form must be used.
- Application must be forwarded directly to the chairman of the Committee.
- If the project is not covered by the Ethical Committee approval for the database (MEDS-online), the applicant must obtain local approval and approval for each of the participants.

- Review will be performed by the committee (at least 2 independent reviewers) within 4 weeks.
- The result of the review will be forwarded to the chairman and the board. The board will send its final decision to the chairman of the committee and the applicant within 2 weeks after the receipt of the reviewers' comment
- Revisions can be suggested to applicants.
- The advancement of the projects should be provided every 6 months to the chairman of Clinical or Research committees.
- The chairman of the committee must inform the EUSTAR chairman of the advancement of the work.
- The advancement of the project will be presented each year at the business meeting.
- A project has to move forward and may be closed if nothing is moving including publication.
- Submission of abstracts must be validated by the Board or by the chairman in case it is later.
- Publications must fulfill the rules of authorship and must be sent to the board before submission.
- Manuscripts must be submitted to the ESCCA chairman and EULAR Steering Committee before submission.
- EULAR meeting and Annals of the Rheumatic Diseases should be first targeted for presentation of the results
- For any information, contact always the chairmen of the Committees.

c. Rules about SSc biospecimens (including DNA)

DNA is property of the patient and of the EUSTAR centre that has delivered it. If other experiments than that included in the EUSTAR project in its approved version are considered

a simple but formal request should be mailed to the committee in charge of the project (basic or clinical) to obtain permission and thereafter to the co-investigators taking care in particular of ethical issues.

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